Asian Journal of Chemistry; Vol. 34, No. 12 (2022), 3251-3256

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23992

Green Synthesized Biogenic Silver Nanoparticles using Leaf Extract of *Syzygium aqueum* (Water Rose Apple) Functionalized by Polymer and their Antibacterial and Antioxidant Activities

CHIRAG MAKVANA*, FARUK ARODIYA and KOKILA PARMAR

Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, India

*Corresponding author: E-mail: chiragmakvana143@gmail.com

Received: 11 July 2022; *Accepted*: 9 October 2022; *Published online*: 25 November 2022; AJC-21052

In this work, silver nanoparticles (size ~8.75 nm) synthesized from the aqueous extract of *Syzygium aqueum* (water rose apple) leaves and were encapsulated in polyvinyl pyrrolidone (PVP). The biosynthesized PVP-silver nanoparticles were characterized by UV-visible, FT-IR, XRD, HR-TEM and SAED techniques. The UV-visible and FT-IR confirmed the successful encapsulation of PVP onto AgNPs. The morphology of PVP-AgNPs was derived by HR-TEM and SAED. As an antibacterial agent, synthesized AgNPs and PVP-AgNPs were tested against *B. subtilis*, *S. aureus*, *S. typhi* and *E. coli*, and proved to be effective against all of them.

Keywords: *Syzygium aqueum*, Biosynthesis, Silver nanoparticles, Encapsulation, Polyvinyl pyrrolidone.

INTRODUCTION

The term "green synthesis" refers to the use of naturally occurring organisms like bacteria, fungi, plants, actinomycetes, *etc*. in the biosynthesis of nanoparticles to produce particles, which are safe for the environment [1,2]. A number of approaches are available for the synthesis of silver nanoparticles *viz.* a photochemical method [3], thermal decomposition of silver compounds [4], electrochemical method [5] and green chemistry routes [6,7]. It appears that several methods for synthesizing nanoparticles are both expensive and selective in their usage of potentially harmful chemicals. These hazardous chemicals may cause contamination on the surface of the nanoparticles similar to the adverse effects of metals in applications [8].

The biosynthesis of nanoparticles has gained a lot of interest as a means of materials synthesis and for good reasons as it doesn't harm the environment [9]. Plant leaves and seed extracts contain reducing sugars (aldoses), terpenoids, amino acids and other chemical substances, which are essential for changing nanoparticle formation [10]. Several metal nanoparticles such as gold, silver, zinc, iron, *etc*. have been synthesized easily by adopting a green approach [11]. The phytocompounds present in the plant extract such as polyols, terpenoids, polyphenols are responsible for metallic ions bioreduction [12-15].

The antimicrobial and multi-drug resistance (MDR) of human pathogens made as problematic issue which needs to discover new natural alternates to overcome this problem [16]. Among the several promising metal nanomaterials, AgNPs seem to be potential antibacterial agents due to their large surface-to-volume ratios and crystallographic surface structure [17]. Moreover, modification of the silver nanoparticles by polymers and surfactants revealed high microbial activity against Gram-negative and Gram-positive bacteria [18]. Polyvinyl pyrrolidone (PVP) is one of the significant capping agents which has been utilized in the nanotechnology field to overcome drawbacks associated with conventional methods of preparation of nanoparticles such as their toxicity, size and agglomeration [19,20]. In literature, PVP has been employed as a capping agent around metal nanoparticles such as iron, silver, gold, zinc, *etc.* [21].

As several biologically active compounds have been isolated from water rose apple (*Syzygium aqueum*) *e.g*. epigallocatechin, epigallocatechin gallate, vescalagin, castalagin and samarangenins A and B [22]. This study focuses on the synthesis, characterization, antibacterial and antioxidant activities of polyvinyl pyrrolidone (PVP) encapsulated AgNPs synthesized using aqueous extract of water apple (*Syzygium aqueum*) leaves.

EXPERIMENTAL

The leaves of *Syzygium aqueum* were collected from local nursery of Patan city, India. All the purchased chemicals were of analytical grade and used as received without further purification. Silver nitrate was purchased from Sigma-Aldrich, USA and polyvinyl pyrrolidone (PVP, *m.w*. 40,000) was purchased from SD Fine Chem. Ltd. Antimicrobial pathogens were obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh, India. Throughout the study, mili-Q water (ultrapure water) was used for the preparation of solutions.

Preparation of leaf extract: Fresh *Syzygium aqueum* leaves were washed thoroughly with deionized water. The leaves were then ground in a grinder after being dried on filter paper. Dried leaves powder was boiled in 100 mL ultrapure water between 60 to 70 ºC. The mixture was cooled and filtered using Whatman filter paper. The obtained yellowish coloured plant extract was stored at -4 ºC for further use.

Synthesis of silver nanoparticles: To Erlenmeyer flask, silver nitrate solution and *Syzygium aqueum* leaves extract were mixed and boiled for 1 h at 60 ºC with constant stirring [23]. After 1 h, the change in colour from a pale yellow colour to a dark brown was observed. The mixture was cooled for 20 min and centrifuged at 10,000 rpm for 20 min at room temperature. To remove any leftover contaminants, the precipitates were washed with deionized water and then dried at 70-75 ºC for 4 h in oven.

Synthesis of PVP encapsulated silver nanoparticles: Polyvinyl pyrrolidone (0.2 g) was dissolved in 100 mL of ultrapure water and stirred for 1 h at 80 ºC. The leaves extract derived AgNPs solution was then gradually added to PVP solution. After 1 h, the colour change was observed from dark brown to light brown. The resultant mixture was centrifuged at room temperature for 15 min at 6000 rpm. Using deionized water, the precipitate was washed and then dried in an oven for 2 h at 70 ºC.

Characterization: The spectra of the synthesized AgNPs and PVP functionalized AgNPs was monitored by a UV-Vis spectrophotometer (Varian Inc., USA) from 300 to 600 nm. The FTIR analysis was performed in the 4000-400 cm^{-1} region with a resolution setting of 5 cm^{-1} to confirm the functional biomolecules associated with the synthesized AgNPs and PVP functionalized AgNPs. XRD analysis was performed to ensure purity using a Rigaku D/max 40 kV X-ray diffraction spectrometer. High resolution transmission electron microscopy (HR-TEM) was used to analyze the structural morphology of the synthesized AgNPs and PVP functionalized AgNPs.

Antimicrobial activity: Biosynthesized AgNPs and PVP-functionalized AgNPs were examined against Gram-positive and Gram-negative bacterial strains. Agar diffusion techniques were used to test the antimicrobial efficacy [24]. In the Petri dish plate, the nutritional agar medium was evenly spread and a 10 mm diameter disc was placed in the centre and employed 100 µL of AgNPs and PVP-AgNPs to test the efficiency of nanoparticles. The culture medium was kept at 37 ºC in an aerobic atmosphere for 24 h. The generation of zones on petri dish plates can be linked to the antimicrobial capabilities of AgNPs and PVP-AgNPs.

Antioxidant properties

DPPH radical scavenging activity: The biosynthesized AgNPs and PVP-AgNPs were evaluated for their antioxidant activities using the DPPH technique [25]. Because of its significant antioxidant capabilities, ascorbic acid was adopted as a standard. For the experiment, ascorbic acid solutions of various concentrations (20, 40, 60, 80, 100, and 120 µg/mL) were prepared. To make DPPH, 20 mg of DPPH was weighed and dissolved in 100 mL of methanol. One mL of DPPH solution was mixed with one mL of biosynthesized AgNPs and PVP-AgNPs and 1 mL of standard ascorbic acid solution and the mixtures were incubated separately for 30 min. The absorbance was measured with a UV-visible spectrophotometer at 517 nm. The free radical scavenging inhibition was calculated using the following formula:

$$\text{Antioxidant activity } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Super oxide anion radical scavenging assay: A mixture of nitroblue tetrazolium (NBT, 1 mM), reduced nicotinamide adenine dinucleotide phosphate (NADPH, 1 mM) and phenazine methosulfate (PMS, 0.1 mM) was incubated for 5 min at room temperature with various quantities of biosynthesized AgNPs and PVP-AgNPs and the absorbance at 560 nm was determined. The percentage of inhibition was calculated by comparing it to a previously determined separate control number. The ability to scavenge was measured using the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is the absorbance of the control and A_s is the absorbance of the sample or standard.

RESULTS AND DISCUSSION

The darkening of the precursor solution to a dark brown colour and the formation of brown precipitation on the inner surface of the reaction flask indicated that the biosynthesis of AgNPs was effective following the addition of *S. aqueum* leaf extract. The colour of a nanoparticles is confirmed by the surface plasmon resonance (SPR).

UV-visible spectroscopy: The stabilizing agent method was followed by a reduction of Ag^+ ions before AgNPs were formed. For biosynthesized AgNPs, a 451 nm band in the UV-visible spectrum was observed in the UV-visible spectrum (Fig. 1a-c). Silver nanoparticles exhibited a surface plasmon peak of 400-500 nm [26]. In the synthesis of biosynthesized AgNPs, the leaf extract of *Syzygium aqueum* acts as a reducing-cum-surface capping agent. While in the PVP encapsulated AgNPs exhibited absorbance peaks range from 447 to 458 nm [27], which are in agreement with the reported research [28].

FTIR studies: In the biosynthesized AgNPs, the key peaks were observed in the FTIR spectra (Fig. 2a-b) at 3410, 3138, 2087, 1632, 1450, 1371, 1089, 837, 646 and 565 cm^{-1}, while in case of synthesized PVP encapsulated AgNPs, the peaks were recorded at 3402, 3224, 2079 and 2378 cm^{-1}. Both AgNPs and PVP-AgNPs have the same C-H bonding vibrational peaks

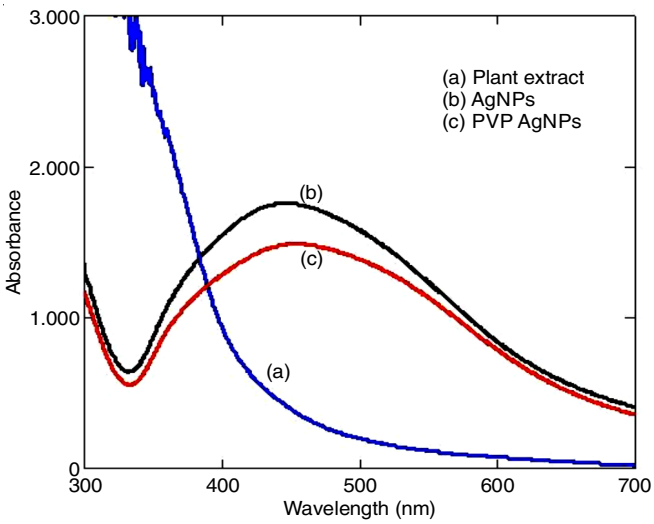

Fig. 1. UV-visible spectrum of (a) plant extract, (b) AgNPs, (c) PVP-AgNPs

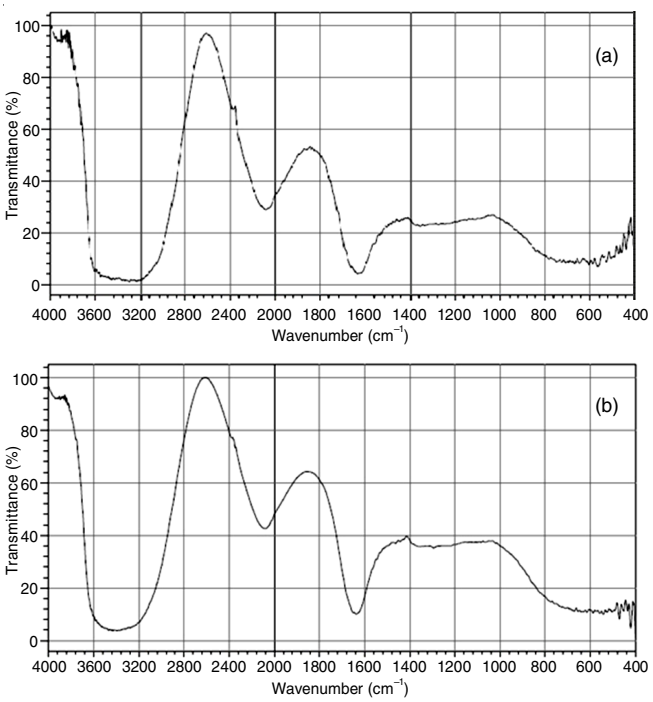

Fig. 2. FT-IR spectrum of (a) AgNPs, (b) PVP-AgNPs

at 1371 and 1361 cm^{-1}. The OH stretching of phenolic groups is associated with the conspicuous band attributed to 3402-3410 cm^{-1}. The peaks at 1450 and 1371 cm^{-1} peaks were due to the N-H stretching of primary amines. Due to the presence of metal carbonyl stretching polymer, the bands at 1632 and 1622 cm^{-1} can be traced to C=O stretching (Fig. 2). When the stretching vibrations associated with –OH and CH/CH_2 groups are integrated with the aliphatic hydrocarbon group in polysaccharide, the proteins and polyphenols are molecules attached to the Ag surface, which is confirmed because of the presence of peaks at 1637 and 1632 cm^{-1} [29-31]. The biosynthesized AgNPs and polymer-capped AgNPs were both produced using an extract from *Syzygium aqueum* leaves as a reducing agent. The results are in line with previous studies [32].

XRD studies: Fig. 3 shows the XRD patterns of biosynthesis of AgNPs and polymeric encapsulated AgNPs. The face-centered cubic (FCC) crystalline structure phase of silver is well-indexed by all diffraction peaks, which are in good accord with JCPDS file no.89-3722. At 27.74º (111), 32.17º (200), 38.09º (220), 36.20º (310), 46.23º (220), 77.41º (311) and 85.57º (322), indicative of significant diffraction peaks were detected. All of the peaks are in the same place, which is consistent with silver. A bioconjugate between the polymer component and the formed polymeric capped AgNPs was modified by the PVP polymer in terms of phase change. The considerable reflection at (111) indicates that nanocrystal growth is almost complete [33]. According to the Debye-Scherrer's equation, the average crystal size of biosynthesized AgNPs was 8.75 nm.

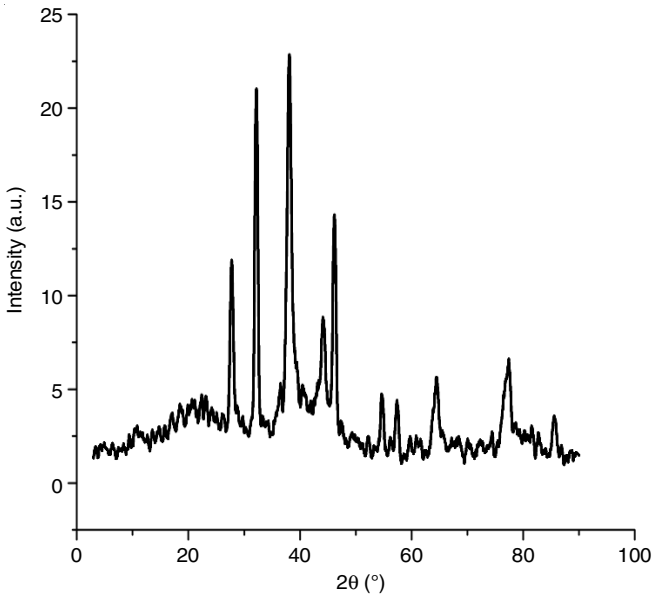

Fig. 3. XRD pattern of PVP AgNPs

HRTEM studies: The spherical, well-spread and homogeneous agglomerated synthesized PVP encapsulated AgNPs particles were in the range of 4 to 13 nm as recorded in the HRTEM image (Fig. 4). The chemically reduced Ag^{o} ions were made zero-valent by coating them with biological molecules (extracted from *Syzygium aqueum* leaf extracts) that contain surface-bound hydroxyl groups [34]. Fig. 4 revealed the ring patterns are shown by the SAED pattern (Fig. 4d). The TEM-derived curve for size distribution is shown in Fig. 5.

Antimicrobial activity: The antibacterial activity of AgNPs synthesized from the water rose apple leaves extract and polymeric encapsulated AgNPs were investigated using a disc diffusion method. The antibacterial activity of PVP-AgNPs was shown to be strong against all the tested bacterial pathogens at 100 µL concentration. As shown in Table-1, the maximum zone of inhibition for *S. typhi* is around 20 mm. For *E. coli*, *B. subtilis* and *S. aureus*, the zone of inhibition was about 18, 17 and 19 mm, respectively. AgNPs in polymer PVP have been proven to be more effective antibacterial agents, since they cause better conformational changes on bacterial cell walls, which increase membrane permeability and lead to bacterial cell death [35].

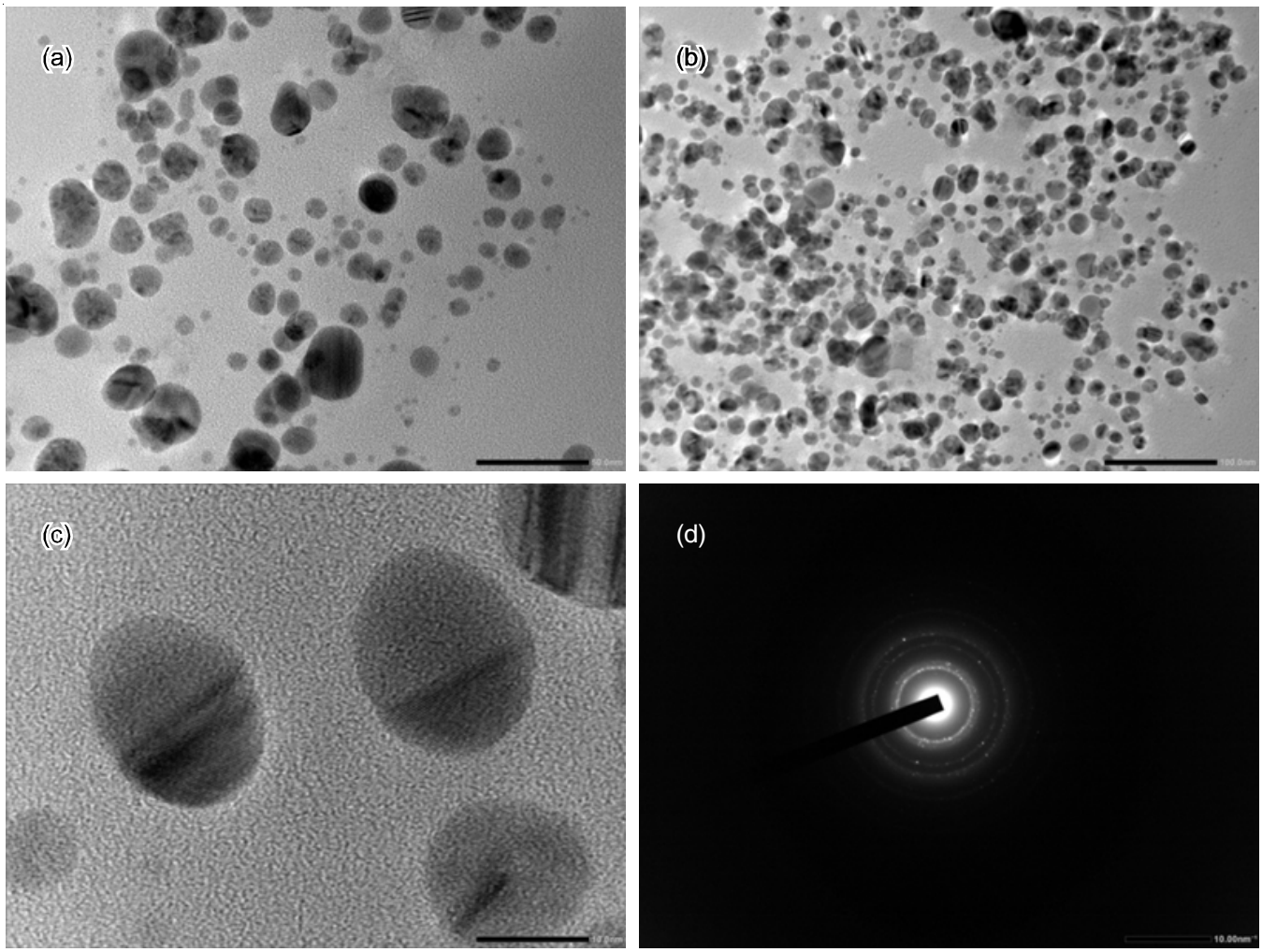

Fig. 4. HR-TEM image of PVP-*b*-Ag NPs observed at 50 nm (a), 100 nm (b), 5 nm (c) and (d) Selected area electron diffraction (SAED) pattern of PVP-*b*-AgNPs

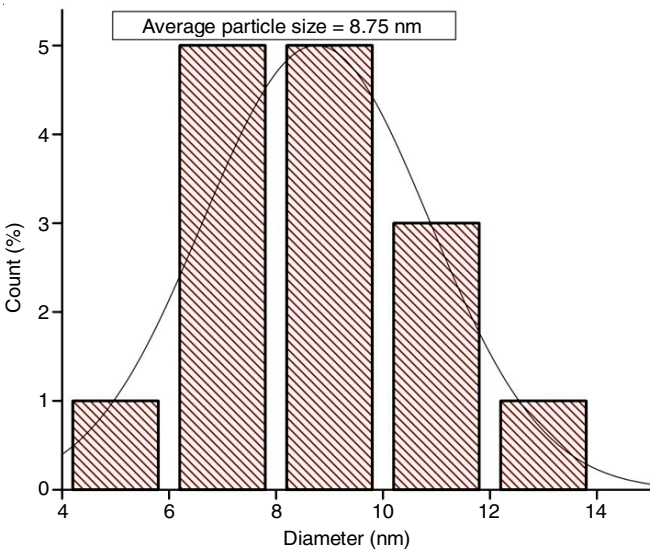

Fig. 5. Size distribution curves from the TEM analysis and SAED pattern of PVP functionalized *b*-AgNPs

Antioxidant properties: Different concentrations of the biosynthesized AgNPs and PVP-capped AgNPs on DPPH radical scavenging activity are illustrated in Fig. 6a. The PVP-capped AgNPs exhibited 52.94% increase in the antioxidant activity at 120 µg/mL, whereas an the same concentration, the standard ascorbic acid demonstrated 50.19% inhibition.

As can be seen in Fig. 6b, the antioxidant scavenging activity using super oxide anion radical scavenging assay was 44.56%, 46.32% and 45.41% exbited by biosynthesized AgNPs, PVP encapsulated AgNPs and ascorbic acid, respectively at the concentration of 120 µg/mL. Moreover, an increase in the concentration of nanoparticles resulted in an increase in the suppression of superoxide.

Conclusion

This study utilized bioorganic components from *Syzygium aqueum* leaves as potential reducing and stabilizing agents for the synthesis of silver nanoparticles (AgNPs). Moreover, in order to enhance the biocompatibility of AgNPs, the polyvinyl pyrrolidone (PVP) encapsulated nanoparticles were also synthesized. The encapsulated PVP onto AgNPs was confirmed by analytical techniques *viz*. UV-vis, FTIR, XRD, HR-TEM and SAED. The size of PVP encapsulated silver nanoparticles was found to between 4 to 13 nm. When compared

TABLE-1
ANTIBACTERIAL ACTIVITY OF PLANT EXTRACT, SILVER NANOPARTICLES OF *Syzygium aqueum* LEAVES AND PVP CAPPED SILVER NANOPARTICLES

Test sample	Concentration (µL)	Inhibition zone (mm)			
		E. coli	*S. typhi*	*B. subtilis*	*S. aureus*
Plant extract	100	15	18	15	15
Penicillin	100	13	16	14	16
AgNPs	100	17	18	16	18
PVP-AgNPs	100	18	20	17	19

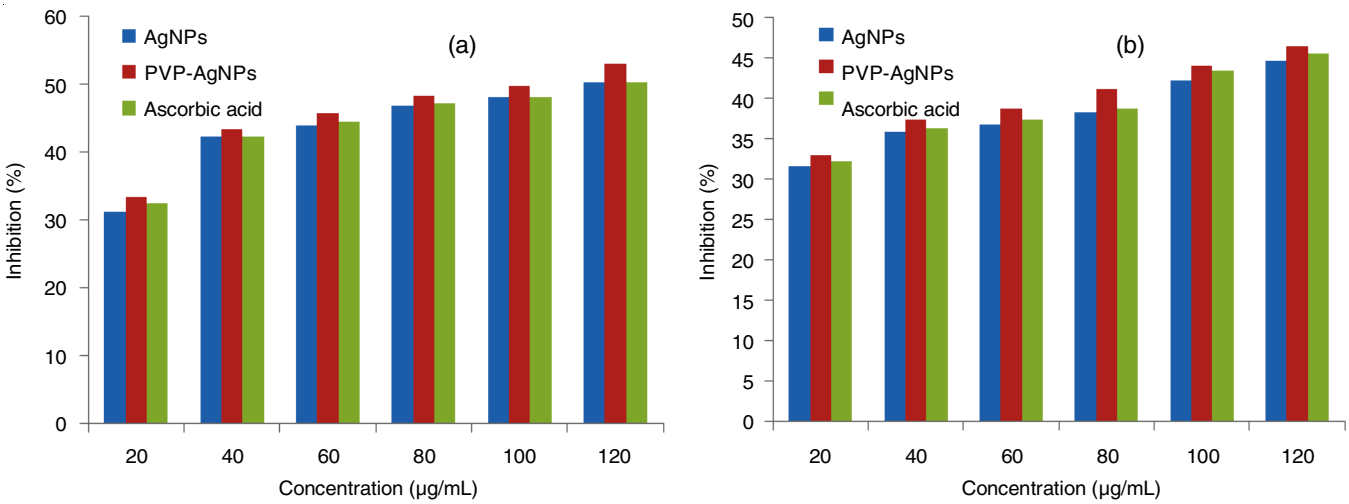

Fig. 6. (a) DPPH radical scavenging activity and (b) super oxide anion radical scavenging assay

to traditional antibacterial medications, the PVP-AgNPs demonstrated greater sensitivity for *S. typhi* than *E. coli, S. aureus* and *B. subtilis*. According to the present observations, synthesized PVP encapsulated AgNPs were found to be better choice as antibacterial and antioxidant agent as compared to neat AgNPs in the antimicrobial activities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M. Shah, D. Fawcett, S. Sharma, S.K. Tripathy and G.E. Jai Poinern, *Materials*, **8**, 7278 (2015); https://doi.org/10.3390/ma8115377
2. H. Chopra, S. Bibi, I. Singh, M.M. Hasan, M.S. Khan, Q. Yousafi, A.A. Baig, M.M. Rahman, F. Islam, T.B. Emran and S. Cavalu, *Front. Bioeng. Biotechnol.*, **10**, 874742 (2022); https://doi.org/10.3389/fbioe.2022.874742
3. X. Lin, F. Rong, D. Fu and C. Yuan, *Powder Technol.*, **219**, 173 (2012); https://doi.org/10.1016/j.powtec.2011.12.037
4. S. Navaladian, B. Viswanathan, R.P. Viswanath and T.K. Varadarajan, *Nanoscale Res. Lett.*, **2**, 44 (2007); https://doi.org/10.1007/s11671-006-9028-2
5. L. Rodriguez-Sanchez, M.C. Blanco and M.A. López-Quintela, *J. Phys. Chem. B*, **104**, 9683 (2000); https://doi.org/10.1021/jp001761r
6. L. Lin, W. Wang, J. Huang, Q. Li, D. Sun, X. Yang, H. Wang, N. He and Y. Wang, *Chem. Eng. J.*, **162**, 852 (2010); https://doi.org/10.1016/j.cej.2010.06.023
7. M. Du, G. Zhan, X. Yang, H. Wang, W. Lin, Y. Zhou, J. Zhu, L. Lin, J. Huang, D. Sun, L. Jia and Q. Li, *J. Catal.*, **283**, 192 (2011); https://doi.org/10.1016/j.jcat.2011.08.011
8. M. Khan, M.S.A. Khan, K.K. Borah, Y. Goswami, K.R. Hakeem and I. Chakrabartty, *Environ. Adv.*, **6**, 100128 (2021); https://doi.org/10.1016/j.envadv.2021.100128
9. K.S. Mukunthan, E.K. Elumalai, T.N. Patel and V.R. Murty, *Asian Pac. J. Trop. Biomed.*, **1**, 270 (2011); https://doi.org/10.1016/S2221-1691(11)60041-5
10. M. Yilmaz, H. Turkdemir, M.A. Kilic, E. Bayram, A. Cicek, A. Mete and B. Ulug, *Mater. Chem. Phys.*, **130**, 1195 (2011); https://doi.org/10.1016/j.matchemphys.2011.08.068
11. D. Zhang, X.-L. Ma, Y. Gu, H. Huang and G.-W. Zhang, *Front. Chem.*, **8**, 799 (2020); https://doi.org/10.3389/fchem.2020.00799
12. C.F. Carolin, P.S. Kumar, A. Saravanan, G.J. Joshiba and M. Naushad, *J. Environ. Chem. Eng.*, **5**, 2782 (2017); https://doi.org/10.1016/j.jece.2017.05.029
13. J. Singh, T. Dutta, K.H. Kim, M. Rawat, P. Samddar and P. Kumar, *J. Nanobiotechnology*, **16**, 84 (2018); https://doi.org/10.1186/s12951-018-0408-4
14. M. Ovais, A.T. Khalil, N.U. Islam, I. Ahmad, M. Ayaz, M. Saravanan, Z.K. Shinwari and S. Mukherjee, *Appl. Microbiol. Biotechnol.*, **102**, 6799 (2018); https://doi.org/10.1007/s00253-018-9146-7
15. Y. Wang, D. O'Connor, Z. Shen, I.M. Lo, D.C. Tsang, S. Pehkonen, S. Pu and D. Hou, *J. Clean. Prod.*, **226**, 540 (2019); https://doi.org/10.1016/j.jclepro.2019.04.128
16. Z. Haghighi Pak, H. Abbaspour, N. Karimi and A. Fattahi, *Appl. Sci.*, **6**, 69 (2016); https://doi.org/10.3390/app6030069
17. X.F. Zhang, Z.G. Liu, W. Shen and S. Gurunathan, *Int. J. Mol. Sci.*, **17**, 1534 (2016); https://doi.org/10.3390/ijms17091534
18. V.K. Sharma, R.A. Yngard and Y. Lin, *Adv. Colloid Interface Sci.*, **145**, 83 (2009); https://doi.org/10.1016/j.cis.2008.09.002
19. M. Goodarz Naseri, E. Saion and N. Khalil Zadeh, *Int. Nano Lett.*, **3**, 19 (2013); https://doi.org/10.1186/2228-5326-3-19

20. G. Pandey, S. Singh and G. Hitkari, *Int. Nano Lett.*, **8**, 111 (2018); https://doi.org/10.1007/s40089-018-0234-6
21. S. Ahlberg, A. Antonopulos, J. Diendorf, R. Dringen, M. Epple, R. Flöck, W. Goedecke, C. Graf, N. Haberl, J. Helmlinger, F. Herzog, F. Heuer, C. Johannes, S. Kittler, M. Köller, K. Korn, W.G. Kreyling, S. Hirn, F. Krombach, J. Lademann, K. Loza, E.M. Luther, M. Malissek, M.C. Meinke, D. Nordmeyer, A. Pailliart, J. Raabe, F. Rancan, B. Rothen-Rutishauser, E. Rühl, C. Schleh, A. Seibel, C. Sengstock, L. Treuel, A. Vogt, K. Weber and R. Zellner, *Beilstein J. Nanotechnol.*, **5**, 1944 (2014); https://doi.org/10.3762/bjnano.5.205
22. G. Nonaka, Y. Aiko, K. Aritake and I. Nishioka, *Chem. Pharm. Bull.*, **40**, 2671 (1992); https://doi.org/10.1248/cpb.40.2671
23. A.A.R. Hamed and S.E. Mustafa, *Biofarmasi J. Nat. Prod. Biochem.*, **16**, 1 (2018); https://doi.org/10.13057/biofar/f160101
24. N.M. Zain, A.G.F. Stapley and G. Shama, *Carbohydr. Polym.*, **112**, 195 (2014); https://doi.org/10.1016/j.carbpol.2014.05.081
25. S. Bhakya, S. Muthukrishnan, M. Sukumaran and M. Muthukumar, *Appl. Nanosci.*, **6**, 755 (2016); https://doi.org/10.1007/s13204-015-0473-z
26. M. Kumara Swamy, K.M. Sudipta, K. Jayanta and S. Balasubramanya, *Appl. Nanosci.*, **5**, 73 (2015); https://doi.org/10.1007/s13204-014-0293-6
27. W. Tiyaboonchai, *Naresuan Univ. J. Sci. Technol.*, **11**, 51 (2013).
28. J.M. Ashraf, M.A. Ansari, H.M. Khan, M.A. Alzohairy and I. Choi, *Sci. Rep.*, **6**, 20414 (2016); https://doi.org/10.1038/srep20414
29. R.D. Rivera-Rangel, M.P. González-Muñoz, M. Avila-Rodriguez, T.A. Razo-Lazcano and C. Solans, *Colloids Surf. A Physicochem. Eng. Asp.*, **536**, 60 (2018); https://doi.org/10.1016/j.colsurfa.2017.07.051
30. D. Philip, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **78**, 327 (2011); https://doi.org/10.1016/j.saa.2010.10.015
31. S. Barua, R. Konwarh, S.S. Bhattacharya, P. Das, K.S.P. Devi, T.K. Maiti, M. Mandal and N. Karak, *Colloids Surf. B Biointerfaces*, **105**, 37 (2013); https://doi.org/10.1016/j.colsurfb.2012.12.015
32. A.K. Mittal, J. Bhaumik, S. Kumar and U.C. Banerjee, *J. Colloid Interface Sci.*, **415**, 39 (2014); https://doi.org/10.1016/j.jcis.2013.10.018
33. T. Wang, X. Jin, Z. Chen, M. Megharaj and R. Naidu, *Sci. Total Environ.*, **466-467**, 210 (2014); https://doi.org/10.1016/j.scitotenv.2013.07.022
34. B. Kumar and K. Smita, Eds.: S. Ranjan, N. Dasgupta and E. Lichtfouse, Phytochemically Functionalized Silver and Gold Nanoparticles to Treat Microbes, Viruses and Cancer, In: Nanoscience in Food and Agriculture 2, Sustainable Agriculture Reviews, Springer International Publishing, Switzerland (2016), vol. 21, Chap. 7, pp. 235-252 (2016).
35. J. Nordberg and E.S. Arnér, *Free Radic. Biol. Med.*, **31**, 1287 (2001); https://doi.org/10.1016/S0891-5849(01)00724-9